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Fluorescence properties and sequestration of peripheral anionic site specific ligands in bile acid hosts: Effect on acetylcholinesterase inhibition activity

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ABSTRACT

The increase in fluorescence intensity of model acetyl cholinesterase (AChE) inhibitors like propidium iodide (PI) and ethidium bromide (EB) is due to sequestration of the probes in primary micellar aggregates of bile acid (BA) host medium with moderate binding affinity of ca. 10^2–10^3 M^{-1}. Multiple regression analysis of solvent dependent fluorescence behavior of PI indicates the decrease in total nonradiative decay rate due to partial shielding of the probe from hydrogen bond donation ability of the aqueous medium in bile acid bound fraction. Both PI and EB affects AChE activity through mixed inhibition and consistent with one site binding model; however, PI (IC_{50} = 20 ± 1 μM) shows greater inhibition in comparison with EB (IC_{50} = 40 ± 3 μM) possibly due to stronger interaction with enzyme active site. The potency of AChE inhibition for both the compounds is drastically reduced in the presence of bile acid due to the formation of BA-inhibitor complex and subsequent reduction of active inhibitor fraction in the medium. Although the inhibition mechanism still remains the same, the course of catalytic reaction critically depends on equilibrium binding among several species present in the solution; particularly at low inhibitor concentration. All the kinetic parameters for enzyme inhibition reaction are nicely correlated with the association constant for BA-inhibitor complex formation.

1. Introduction

Alzheimer's disease (AD) is a chronic and progressive neurodegenerative disorder. The symptoms include confusion, irritability and aggression, mood swings, language breakdown, long-term memory loss, and the general withdrawal of the sufferer as their senses decline. The AD patients suffer from insufficient amount of acetylcholine (ACh), the primary neurotransmitter released in the muscular synaptic junctions, mostly due to the loss of cholinergic neuron in the hippocampus and in the cortex. This situation is further aggravated by the presence of an enzyme called acetylcholinesterase (AChE) in the synapse that degrades ACh into choline and acetate [1,2]. To ameliorate the problem in AD, AChE inhibitors are being used clinically in a moderate amount [3,4]. These are essentially a class of chemicals which can take the place of ACh at the active site of AChE, significantly reducing the neurotransmitter's ability to bind with AChE and therefore, limiting the rate at which ACh can be broken down. The result is the presence of sustained amount of ACh at post-synaptic receptor molecules and, subsequently, a continuous firing of action potentials. Interestingly, a wide range of compounds show this inhibitory activity mainly through binding with AChE at a number of sites, including the catalytic triad (Ser203, His447, Glu334), neighboring hydrophobic residues (Trp86, Tyr133, Tyr337, and Phe338), the acyl pocket (Phe295 and Phe297), or a peripheral binding site (Tyr72, Tyr124, Glu285, Trp286, Tyr341, and Asp74) [5,6].

Ethidium bromide (EB) and propidium iodide (PI) are well known DNA intercalators and frequently used as cell staining media due to their large increase in fluorescence intensity upon binding with nucleotides [7–9]. Both the compounds (Chart 1, for structure) contain identical aromatic phenanthridinine planar rings; the only difference is the presence of a linear side chain containing quaternary amine group in the case of PI, thereby making this molecule doubly charged. In addition to their use as fluorescent bio-markers, both EB and PI show interesting photo-physical behavior due to strong modulation in their fluorescence properties in the presence of different micro-heterogeneous environments [10–14]. Further, both EB and PI are reported to be potent cholinergic inhibitors due to their specific interaction at the peripheral anionic site (PAS) of the AChE active pocket [15–18].

Research in the field of bile acids (BAs) has continued to receive considerable interest in recent times from both their biochemistry and physiology [19–21]. BAs are steroid acids found predominantly in the bile of mammals. Bile acids aid in fat absorption and modulate cholesterol levels. They are produced from cholesterol in the liver and are stored in the gall bladder. There are quite a few approved orally administered cholinergic AD drugs like tacrine, donepezil, galantamine and

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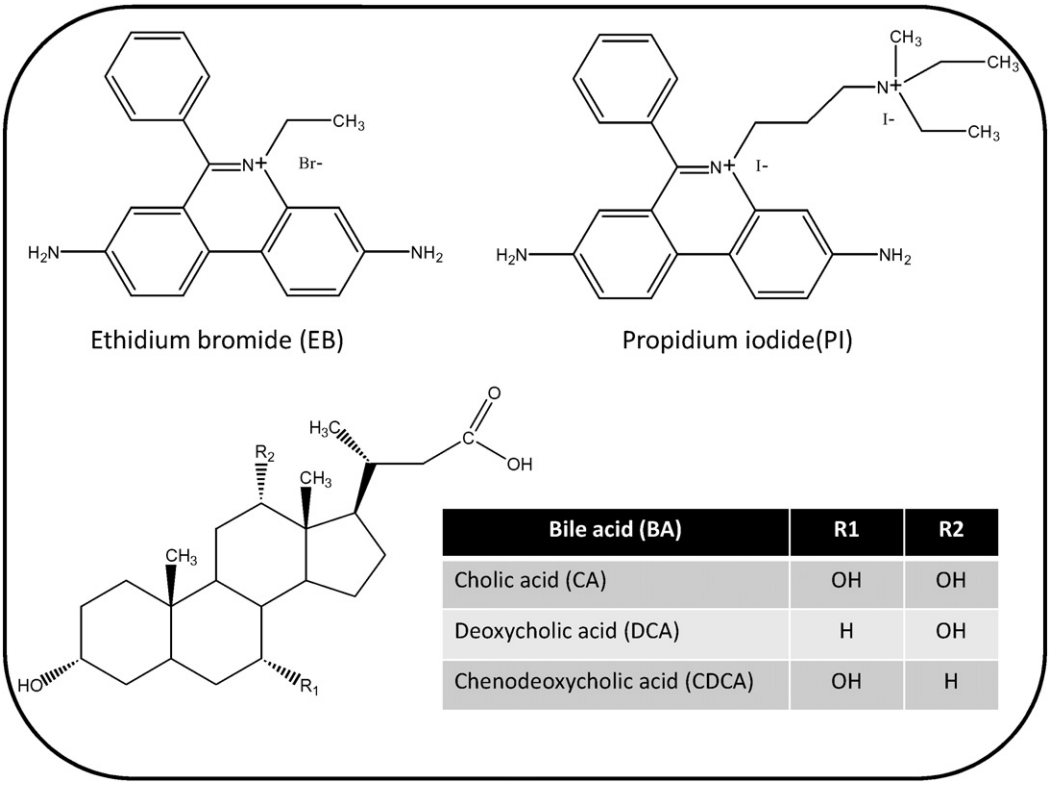

Bile acid (BA)	R1	R2
Cholic acid (CA)	OH	OH
Deoxycholic acid (DCA)	H	OH
Chenodeoxycholic acid (CDCA)	OH	H

Chart 1. Structure of the peripheral anionic site specific AChE inhibitors, propidium iodide (PI) and ethidium bromide (EB), used in this study. Structural representations of three bile acids (BA) are also shown in the lower panel.

rivastigmine. However, except for tacrine which is known to be associated with mild or moderate liver dysfunction after drug administration [22,23], the others used for AD treatment are rarely known to affect acute liver injury and have a low rate of associated serum enzyme elevations [24]. Nevertheless, it is interesting to know the extent to which the cholinergic inhibitor activity is modulated in the presence of bile acid hosts. In fact, clinical observation is already available to prove the reduced bioavailability of rivastigmine drug towards AChE inhibition to almost 35% of the administered dose [25]. Recently, we have reported the fluorescence modulation and binding behavior of EB in three major human BAs; the primary types consisting of cholic acid (CA), chenodeoxycholic acid (CDCA), and also one of the secondary types, namely deoxycholic acid (DCA) [26]. In the present contribution we have extended our investigation to see the sequestration and fluorescence spectral modulatory behavior of PI, the higher homolog of EB, in BA medium by steady state and time-resolved fluorescence experiments. Also, the effect of both these probes confiscated in the hydrophobic domain of BA hosts on the AChE inhibition kinetics was investigated in detail.

2. Experimental Details

2.1. Reagents & Chemicals

Lyophilized powder form of propidium iodide (PI) was pursued from Aldrich Chemical Company (product no. P4170) and was used without any further purification; whereas, analytical grade ethidium bromide (EB) was procured from Sisco Research Laboratories (SRL), India (product no. 054,817) and the purity was checked by chromatographic techniques before use. The type V–S lyophilized powder, activity ≥1000 units/mg protein form of acetylcholinesterase from *Electrophorus electricus* (electric eel) and acetylthiocholine iodide obtained from Sigma Aldrich (product no. C2888 and A5751, respectively) were used for activity measurements. The extra-pure powder form of Dithiobis-(2-nitrobenzoic acid) (DTNB) (Ellman's Reagent) and sodium bicarbonate were obtained from Sisco Research Laboratories (SRL), India (product no. 044883 and 1944142, respectively). The gelatin and pH 9.2 buffer tablets were received from Qualigens fine chemicals (a division of GlaxoSmithkline Pharmaceuticals Ltd.), India. The 1% (v/v) gelatin solution was used to make the enzyme stock solution. The solution pH was checked with Systronics μ-pH system 361. All the organic solvents were of spectroscopic grade (>99.5%) as received from Alfa Aesar and, in some cases, from Aldrich Chemical Company. The characteristic solvent parameters are listed in Table 1. The bile acids viz. cholic acid (CA) deoxycholic acid (DCA) and chenodeoxycholic acid (CDCA) were obtained from Sigma-Aldrich (product nos. C1129, C9377 and C9377, respectively). The water used

Table 1
Solvent parameters.

No. of solvent	Δf (ε, n)[a]	E_T (30)[b]	α[c]	β[c]	π*[c]
1. Tetrahydrofuran	0.21	37.4	0	0.55	0.58
2. 1-Butanol	0.26	49.7	0.84	0.84	0.47
3. Dimethyl sulfoxide	0.26	45.1	0	0.76	0.1
4. Dimethyl formamide	0.27	43.2	0	0.69	0.88
5. 1-Propanol	0.27	50.7	0.84	0.9	0.52
6. Isopropyl alcohol	0.27	48.4	0.76	0.95	0.48
7. Acetone	0.28	42.2	0.08	0.48	0.71
8. Acetonitrile	0.3	45.6	0.19	0.4	0.75
9. Methyl alcohol	0.31	55.4	0.98	0.66	0.6
10. Water	0.32	63.1	1.17	0.47	1.09

[a] Polarity parameter ($= \frac{\varepsilon-1}{2\varepsilon+1} - \frac{n^2-1}{2n^2+1}$) where, solvent dielectric constant and refractive indices are represented by ε and *n*, respectively.
[b] Reichardt solvent parameter.
[c] Kamlet–Taft solvent parameters.